

Quercetin and Vincristine Potential of Methanolic Extract from Arjun Tree

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Abstract

Polyphenolic compounds like Gallic acid, ethyl gallate, quercetin, kempferol, proanthocyanidin, EPGA, resveratrol have been familiar as nutraceuticals for possessing anti-oxidant, anti-inflammatory, and anti-cancer activities. Arjun Tree (*Terminalia Arjuna*) is a very ancient plant which contains many reported polyphenolic compounds having antifungal, cardioprotective, anti-mutagenic, anti-inflammatory, anti-allergic, anti-bacterial, cytotoxic, anti-oxidant properties. Here we reported methanolic extract of *Terminalia arjuna*, which neutralized 50% (IC₅₀) of free radicals at a concentration of 3.29 µg/ml, while the standard neutralizes 50% (IC₅₀) of free radical at a concentration of 1.92 µg/ml. The total phenolic content was 449.8 µg/ml of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentration was 206.91 µg/ml, expressed as quercetin equivalents. In the brine shrimp lethality test, LC₅₀ obtained from the line slope were 0.419 and 12.5, which is greater than that of 0.781 µg/ml for standard vincristine sulphate, aqueous fraction.

Keywords: Polyphenolic Compounds, Methanolic Extract, Antioxidant, Cytotoxicity.

Introduction

Terminalia arjuna is the plant that belongs to the genus *Terminalia*, which belongs to the Combretaceae family and this plant is well distributed to the tropic and subtropics like warm temperature regions like Bangladesh, India, Srilanka etc. (Exell & Stace, 1966; Heywood et al. 2007). This plant is also known as koha or white marudah and it is used for many medicinal properties. One research work reported that the antioxidant action of aqueous extract of *T. arjuna*, which may play a role in the anti-carcinogenic activity by reducing the oxidative stress along with inhibition of anaerobic metabolism (Verma N., Vinayak M. 2009). There is no report of methanolic extract of this plant in respect of Polyphenolic compound like quercetin and anti-cancer drug like vincristine. However, phytochemical investigation revealed the anti-oxidant activity of the bark extract from *Terminalia arjuna* and its lethal bioassay test of two fraction (aqueous and ethyl acetate) for determining the cytotoxic activity of this plant.

Plant material

Plant sample of *Terminalia arjuna* was collected from Dhaka in November 2017 from Dr. Md. Abdul Muhit, Assistant Professor,

Department Of Clinical Pharmacy & Pharmacology, University Of Dhaka. Ref.No, CPP/DU/01-2016-TA.

Method

Bark of the plant was firstly separated from the plant. Then it was cut into small pieces and air dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature (not more than 50°C) to effect grinding. It was then ground into coarse powder. The coarse powder was then in air – tight container and kept in cool, dark and dry place for further use.

The air dried and powdered plant material (500g) was submerged with methanol(2 litres) in an air tight, clean flat bottomed container for 15 days at room temperature with occasional stirring and shaking. It was then filtered through a fresh cotton plug and finally with a man N0.1 filter paper. The volume of the filtrate was then reduced using a rotary evaporation at low temperature and pressure. The weight of the crude extract was 36.45 gm.

Solvent – solvent partitioning

Solvent solvent partitioning was done using the protocol of the standard laboratory. The crude extract was dissolved in 600ml of

water. Then gently added 300 ml of Ethyl acetate for 4 times. The ethyl acetate layer was collected and the fraction of this was 10 g. Then the water layer fraction was collected about 19 g.

Result

Methanolic extracts were prepared to examine the total phenolic content, flavonoid concentration and antioxidant activity.

A.1 Total Phenolic count

The total phenolic contents in the examined extracts was 449.8 mg GA/total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GA/g of extract) extract mg of GA/g of extract (X = 449.8 mg/ml).

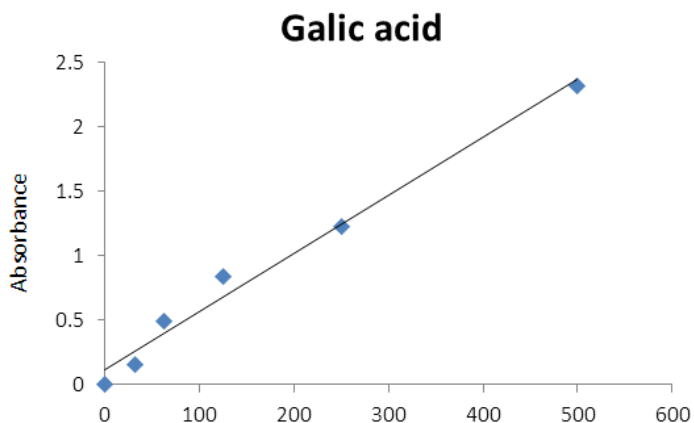


Figure 1: Standard curve of Gallic acid for determination of phenolic count.

Total Flavonoid count

The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: $y = 0.0067x + 0.0132$, $r^2 = 0.999$), mg of Qu/g of extract The concentration of flavonoids in plant extracts was 206.91 mg/g in methanolic extract). Concentrations of flavonoids in the plant extracts expressed in terms of quercetin equivalent (mg of Qu/g of extract) extract mg of Qu/g of extract (X = 206.91 mg/ml).

Anti-oxidant activity

The capacity to neutralize DPPH radicals was found for methanolic extract which neutralized 50% (IC50) of free radicals at the concentration of 3.29 µg/ml, while the standard neutralizes 50% (IC50) of free radical at the concentration of 1.92 µg/ml. IC50 of Ascorbic acid is 1.92 µg/ml, where the IC50 of Terminalia Arjuna crude is 3.29 µg/ml.

Table 1.1: % of free radical scavenging capacity of Ascorbic acid and crude extract.

No.	Ascorbic acid			Crude extract		
	Conc (µg/ml)	Abs	% inhibit	Conc. (µg/ml)	Abs	% inhibit
1	500	0.054	87	500	0.065	84
2	250	0.055	86	250	0.070	83
3	125	0.055	86	125	0.078	81
4	62.5	0.056	86	62.5	0.081	80
5	31.25	0.058	85	31.25	0.082	80
6	15.62	0.058	85	15.62	0.088	78
7	7.81	0.059	85	7.81	0.093	77
8	3.90	0.065	84	3.90	0.168	59
9	1.95	0.201	51	1.95	0.235	43
10	0.967	0.312	25	0.967	0.368	11

Brine shrimp lethality bioassay

This method is done to determine the cytotoxicity of the test samples from Terminalia Arjuna and their effectiveness on the cell to destroy them successfully.

Table B.2: Results of the test samples of Terminalia Arjuna

Sample	LC50(ug/ml)	Regression equation	R ²
Vincristine Sulphate (std)	0.419	Y = 30.404x + 61.071	0.9476
MeOH crude	12.5	Y = 0.2517x + 0.196	0.8951
Aqueous fraction	> 0.781	Y = 0.143x + 0.6917	0.844
Ethyl acetate fraction	> 0.781	Y = 0.0685x + 0.8546	0.7961

Table B. 3: Effects of methanolic crude and it's aqueous fraction on brine shrimp neuplii.

Conc. (µg/ml)	LogC	%Mortality		LC ₅₀ (µg/ml)		Vincristine sulphate			
		C	AF	C	AF	Conc.(µg/ml)	Log C	%Mortality	LC ₅₀ µg/ml
400	2.602	100	100	12.5	> 0.781	40	1.602	100	0.419
200	2.301	80	100			20	1.301	100	
100	2	60	100			10	1.000	90	
50	1.699	50	100			5	0.698	90	
25	1.398	50	100			2.5	0.397	80	
12.5	1.097	50	80			1.25	0.096	70	
6.25	0.796	40	80			0.625	-0.204	60	
3.125	0.495	30	70			0.3125	-0.505	50	
1.563	0.194	30	70			0.15625	-0.806	30	
0.781	-0.107	20	70			.078125	-1.107	20	

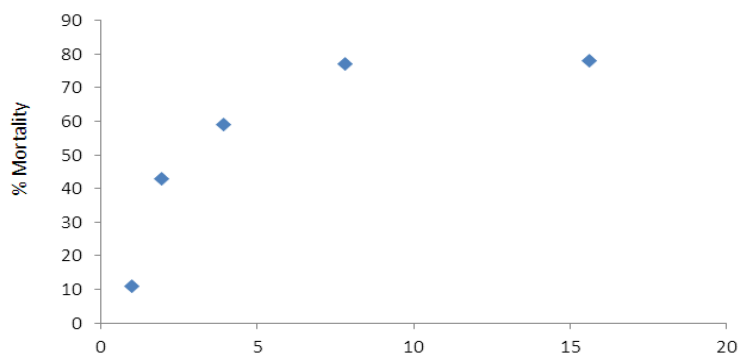


Figure B.1: Effect of Vincristine sulphate on brine shrimp neuplii.

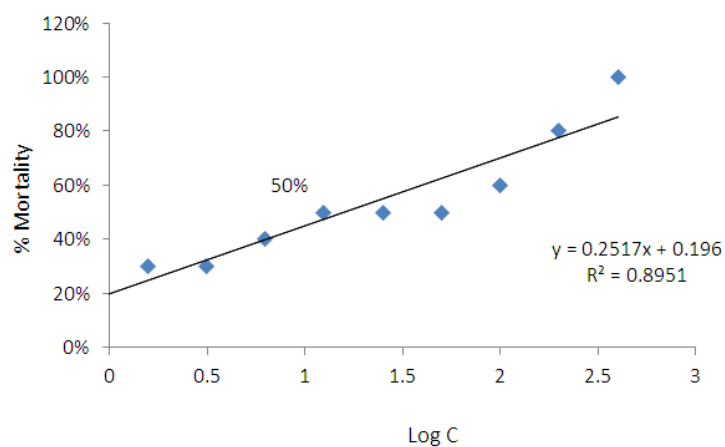


Figure B.2: Effect of Crude sample on brine shrimp neuplii.

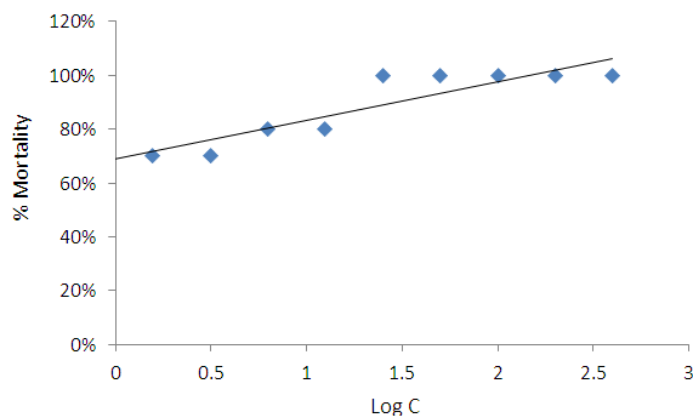


Figure B.3: Effect of Aqueous soluble partition ate of methanolic extract on brine shrimp neuplii.

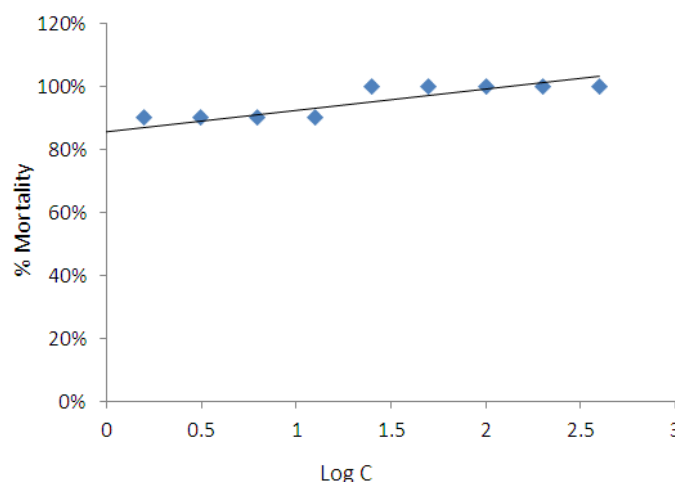


Figure B.4: Effect of Ethyl acetate fraction soluble partition of methanolic extract on brine shrimp neuplii.

Discussion

As we see, the amount of polyphenol is so high (449.8 mg/ml half of the total amount of 1mg crude) and also the flavonoid is (206.91 mg/ml), we can say it is a very good antioxidant property containing plant part whose IC₅₀ value (3.29µg/ml) is also very good like standard (1.92µg/ml).

The degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (0.781 µg/ml) to highly significant with the highest concentration (400µg/ml). Maximum mortalities took place at a concentration of 400 µg/ml, whereas least mortalities were at 0.781 µg/ml concentration. In other words, mortality increased gradually with the increase in concentration of the test samples.

LC₅₀ obtained from the best line slope were 0.419, 12.5, >0.781 µg/ml for vincristine, crude and other fractions respectively.

In comparison to positive control (vincristine) the cytotoxicity exhibited by ethyl acetate and aqueous fraction of methanolic extract was highly significant. On the other hand, crude extract demonstrated promising activity.

Conclusion

The methanolic extract of Terminalia arjuna shows a great antioxidant activity almost like the standard ascorbic acid. It also has very good amount of phenol and flavonoid.

In the brine shrimp lethality bioassay, in comparison to positive control (vincristine sulphate) the cytotoxicity exhibited by the ethyl acetate fraction and aqueous fraction of the methanolic extract was highly significant.

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